Performance of the Vitek MS matrix-assisted laser desorption ionization time-of-flight mass spectrometry system for identification of Gram-positive cocci routinely isolated in clinical microbiology laboratories

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We evaluated the performance of the Vitek MS for identification of Gram-positive cocci routinely isolated in clinical microbiology laboratories. With a total of 424 well-characterized isolates, the results of the Vitek MS were compared to those of conventional methods and 16S rRNA gene sequencing. The Vitek MS correctly identified 97.9 % of the isolates tested to species level. The Vitek MS correctly identified the species of 97.2 % of the staphylococci (95.9 % of coagulase-negative staphylococci), 97.8 % of the streptococci, and 100 % of the enterococci. For the identification of Gram-positive cocci isolates, the overall concordance rate between conventional identification and the Vitek MS was 94.5 %. The Vitek MS matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) system can be a reliable and rapid method for the identification of most relevant Gram-positive cocci. In addition, expanding the database of the Vitek MS, especially for coagulase-negative staphylococci, is needed to enhance the performance of the Vitek MS.

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INTRODUCTION

The process of bacterial identification from clinical specimens is a critical step for confirmation and appropriate management of infection. Conventional identification methods are mainly based on phenotypic characteristics such as colony morphology and biochemical reactions. These methods require consecutive steps and are costly and time-consuming, taking at least 12 to 24 h (Seng *et al.*, 2009; Cherkaoui *et al.*, 2010; Dubois *et al.*, 2012). Using the technique of matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS), intact larger biomolecules such as proteins can be analysed (Karas & Hillenkamp, 1988), and MALDI-TOF MS has been proposed for bacterial identification (Claydon *et al.*, 1996). Recently, MALDI-TOF MS has been introduced for the identification of micro-organisms in routine clinical laboratories (Seng *et al.*, 2009). During this process, proteins from micro-organisms are ionized by a laser to generate characteristic mass spectral fingerprint profiles based on their mass-to-charge ratios (Fenselau & Demirev, 2001). Microbial identification is performed by comparison of the protein spectrum generated from intact, whole bacterial cells to a library of species-specific reference protein spectra profiles (Fenselau & Demirev, 2001).

During the past few years, MALDI-TOF MS instruments for microbial identification have been improved, and commercial, easy to use MALDI-TOF MS devices containing their own algorithms and large databases of reference strains have been introduced (Emonet *et al.*, 2010). Many studies have reported the fast, cost-effective and accurate performance of these MALDI-TOF MS systems for the identification of various bacteria and yeast (Bizzini *et al.*,

Abbreviations: CoNS, coagulase-negative staphylococci; MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

2010; Cherkaoui *et al.*, 2010; van Veen *et al.*, 2010; Neville *et al.*, 2011). Representative MALDI-TOF MS instruments include the Biotyper (Bruker Daltonics), Axima Confidence spectrometer (Shimadzu-Biotech Corporation), and the recently launched Vitek MS (bioMérieux). Although better accuracies for Gram-positive cocci have been reported with the Vitek MS, the number of Gram-positive cocci such as streptococci and enterococci were relatively limited in these previous studies (Dubois *et al.*, 2012; Martiny *et al.*, 2012). The objective of the present study was to evaluate the performance of the recently launched MALDI-TOF MS-based Vitek MS system for the identification of Gram-positive cocci routinely isolated in clinical microbiology laboratories.

METHODS

Bacterial isolates and conventional identification. During a 4 month period, isolates of aerobic Gram-positive cocci randomly collected from various clinical specimens, including blood, urine, stool, pus, cerebrospinal fluid, respiratory tracts, wounds, rectal swabs, and catheter tips from three microbiology laboratories (two university-affiliated hospitals and one reference laboratory) were conserved in 10 % skim milk at −80 °C for identification with the Vitek MS. We planned to include similar number of isolates for each species but the number of rare species was limited during the study period. A total of 424 well-characterized isolates were tested in this study: 218 staphylococci belonging to 13 species, 135 streptococci (21 *Streptococcus pneumoniae*, 53 beta-haemolytic streptococci and 61 viridans-group streptococci belonging to eight species), 70 enterococci belonging to six species and one *Abiotrophia defectiva*. Five ATCC reference strains were also tested: ATCC 29212 *Enterococcus faecalis*, ATCC 49619, *S. pneumoniae*, ATCC 29213, *Staphylococcus aureus*, ATCC 12228 *Staphylococcus epidermidis* and ATCC 700329 *Enterococcus casseliflavus*.

Identification of the bacterial isolates was performed with conventional methods using biochemical tests and the Gram-positive identification (GPI) cards of the Vitek 2 system (bioMérieux) and with the Vitek MS. The Vitek 2 identification was performed according to the manufacturer's instructions. Discrepant isolates between the Vitek MS and conventional identification were subsequently identified by 16S rRNA gene sequencing. When the results of conventional identification were not certain or needed confirmation (e.g. *Streptococcus dysgalactiae* subsp. *equisimilis/dysgalactiae*), sequence-based molecular identification was also performed. Concordant results and results confirmed by 16S rRNA gene sequencing were considered as reference identifications.

MALDI-TOF MS identification. All 424 isolates were identified by the Vitek MS system (Vitek MS database version 2 for *in vitro* diagnostic use) according to the manufacturer's instructions. Briefly, a portion of a fresh colony was smeared onto a Vitek MS DS target slide and the preparations were overlaid with 1 μl matrix solution (a saturated solution of α-cyano-4-hydroxycinnamic acid in 50 % acetonitrile and 2.5 % trifluoroacetic acid). After drying, the target plate was loaded into the Vitek MS mass spectrometer and air-dried for 1 to 2 min at room temperature. As a calibration and internal identification control, the *Escherichia coli* ATCC 8739 strain was inoculated on the calibration spots. The 500 shots from different positions of the each spot were collected by the mass spectrometer with the Acquisition Station software package. Generated mass fingerprints were processed by the computer engine, and the advanced spectrum classifier algorithm automatically identified the organism by comparing the obtained peaks (presence and absence of specific peaks) with those of the reference spectrum of each claimed species. A percentage probability (confidence value) was calculated and this number represents the similarity of specific peaks between the generated spectrum and the database spectra. A confidence value of 99.9 % means a perfect match and confidence values of 60 % to 99.8 % indicate spectra that are sufficiently close to that of a reference spectrum. When a single unique pattern was not identified, a list of possible organisms was given ['low discrimination' (LD)] or the strain could not be determined within the scope of the database ('no identification').

In this study, the overall correct identification was defined as including the following levels (Dubois *et al.*, 2012): (i) correct identification to the species level when the system proposed the reference species identification as a single choice or with LD to the subspecies level, (ii) correct identification to the genus level when the system proposed the species identification of the same genera to reference identification as a single choice or with LD results, and (iii) correct identification above the genus level when the system proposed the reference species identification among a set of LD results including species of different genera.

16S rRNA gene sequencing. DNA was extracted from cultured colonies by the boiling method with Chelex® 100 Resin (Bio-Rad Laboratories) and PCR was performed with two pairs of primers which amplify the 16S rRNA gene between positions 8 and 1509 of the *E. coli* 16S rRNA gene (Schuurman *et al.*, 2004). PCR amplification was performed with 35 cycles of the following conditions: denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min and extension at 72 °C for 1 min. Amplified products of the 16S rRNA gene were subjected to 2 % agarose gel electrophoresis, and direct sequencing was performed using an automatic DNA sequencer (ABI 3730XL, Life Technology). The results of 16S rRNA sequencing were analysed using the NCBI GenBank and EzTaxon (www.eztaxon.org, version 2.1) databases according to the Clinical and Laboratory Standards Institute (CLSI) MM18-A (Chun *et al.*, 2007; CLSI, 2008).

RESULTS

Performance of the Vitek MS system compared to reference identifications

All five reference strains were correctly identified by the Vitek MS. The 16S rRNA gene sequencing was performed to solve discrepancies or to obtain a more accurate reference identification for 33 (7.7 %) isolates. The Vitek MS correctly identified 97.9 % (415/424) of the isolates tested at the species level and 98.6 % (418/424) of the isolates tested at least at the genus level. At the species level, the Vitek MS correctly identified 97.2 % of the staphylococci, 97.8 % of the streptococci and 100 % of the enterococci. The overall correct species identification of coagulase-negative staphylococci (CoNS) was 95.9 % (142/148). All isolates of the 70 *S. aureus*, 50 *S. epidermidis*, 21 *S. pneumoniae*, 23 *Streptococcus pyogenes*, 19 *Streptococcus agalactiae*, 39 *E. faecalis*, 23 *Enterococcus faecium* and 1 *A. defectiva* tested were correctly identified using the Vitek MS (Table 1). The Vitek MS gave LD results of *Streptococcus anginosus/constellatus* in two isolates of *S. anginosus* and the single choice of *Staphylococcus capitis* in an isolate of *Staphylococcus pettenkoferi*.

A correct identification to the above genus level was made in two isolates of *Staphylococcus warneri* and a correct reference

Table 1. The Vitek MS results of 424 Gram-positive cocci isolates compared to reference identification

Reference ID*	No.	No. (%) of isolates			
		Correct ID to the level of			No ID
		Species	Genus	Above genus	
Staphylococci	**218**	**212 (97.2)**	**1 (0.5)**	**2 (0.9)**	**3 (1.4)**
Staphylococcus aureus	70	70 (100.0)	–	–	–
Staphylococcus epidermidis	50	50 (100.0)	–	–	–
Staphylococcus capitis	27	26 (96.3)	–	–	1 (3.7)
Staphylococcus hominis	26	26 (100.0)	–	–	–
Staphylococcus haemolyticus	17	17 (100.0)	–	–	–
Staphylococcus warneri	4	2 (50.0)	–	2 (50.0)	–
Staphylococcus lugdunensis	5	5 (100.0)	–	–	–
Staphylococcus cohnii subsp. *urealyticus/cohnii*†	5	5 (100.0)	–	–	–
Staphylococcus saprophyticus	5	5 (100.0)	–	–	–
Staphylococcus caprae	3	3 (100.0)	–	–	–
Staphylococcus simulans	2	2 (100.0)	–	–	–
Staphylococcus sciuri	1	1 (100.0)	–	–	–
Staphylococcus pettenkoferi	3	0 (0.0)	1 (33.3)	–	2 (66.7)
Streptococci	**135**	**132 (97.8)**	**2 (1.5)**	–	**1 (0.7)**
Streptococcus pneumoniae	21	21 (100.0)	–	–	–
Beta-haemolytic streptococci	**53**	**52 (98.1)**	–	–	**1 (1.9)**
Streptococcus pyogenes	23	23 (100.0)	–	–	–
Streptococcus agalactiae	19	19 (100.0)	–	–	–
Streptococcus dysgalactiae ssp. *equisimilis/dysgalactiae*†	11	10 (90.9)	–	–	1 (9.1)
Viridans streptococci	**61**	**59 (96.7)**	**2 (3.3)**	–	–
Streptococcus anginosus	22	20 (90.9)	2 (9.1)	–	–
Streptococcus constellatus	10	10 (100.0)	–	–	–
Streptococcus intermedius	1	1 (100.0)	–	–	–
Streptococcus mitis/oralis†	13	13 (100.0)	–	–	–
Streptococcus sanguis	5	5 (100.0)	–	–	–
Streptococcus parasanguinis	3	3 (100.0)	–	–	–
Streptococcus gordonii	2	2 (100.0)	–	–	–
Streptococcus salivarius	5	5 (100.0)	–	–	–
Enterococci	**70**	**70 (100.0)**	–	–	–
Enterococcus faecalis	39	39 (100.0)	–	–	–
Enterococcus faecium	23	23 (100.0)	–	–	–
Enterococcus casseliflavus	3	3 (100.0)	–	–	–
Enterococcus avium	3	3 (100.0)	–	–	–
Enterococcus hirae	1	1 (100.0)	–	–	–
Enterococcus raffinosus	1	1 (100.0)	–	–	–
Other Gram-positive cocci	**1**	**1 (100.0)**	–	–	–
Abiotrophia defectiva	1	1 (100.0)	–	–	–
Total	**424**	**415 (97.9)**	**3 (0.7)**	**2 (0.5)**	**4 (0.9)**

*Concordant results or results confirmed by 16S rRNA gene sequencing were considered as reference identification (ID).
†The Vitek MS did not differentiate *S. cohnii* subsp. *cohnii* and *S. cohnii* subsp. *urealyticum*, *S. dysgalactiae* subsp. *dysgalactiae* and *S. dysgalactiae* subsp. *equisimilis*, *S. mitis and S. oralis.*

identification (*S. warneri*) was included in multiple choices among a set of LD results, including species of different genera such as *Neisseria gonorrhoeae* and *Prevotella buccalis.* The Vitek MS system gave an absence of identification for four isolates (0.9 %, 4/424) including one *S. capitis*, two *S. pettenkoferi* and one *Streptococcus dysgalactiae* (Table 2).

Comparison of conventional and Vitek MS identifications

In comparing the conventional identification with the Vitek MS, the identification matched at species level in 94.5 % (401/424) of the instances, matched at least genus level in 98.3 % (417/424), and did not match in 1.7 % (7/

Table 2. Analysis of the 23 discrepant results between the conventional method and the Vitek MS

ID, Identification; V, Vitek 2 is correct at species level; M, Vitek MS is correct at species level; B, both incorrect at species level.

16S rRNA sequencing	Conventional method (Vitek 2 system)	Vitek MS	Correct ID
S. caprae/capitis	*Staphylococcus capitis*	No identification	V*
Staphylococcus warneri	*Staphylococcus warneri*	*Staphylococcus warneri/Nesseria gonorrhoeae/ Prevotella buccalis*	V
Staphylococcus warneri	*Staphylococcus warneri*	*Staphylococcus warneri/Prevotella buccalis*	V
Streptococcus anginosus	*Streptococcus anginosus*	*Streptococcus anginosus/constellatus*	V
Streptococcus anginosus	*Streptococcus anginosus*	*Streptococcus anginosus/constellatus*	V
Streptococcus dysgalactiae subsp. *equisimilis*	*Streptococcus dysgalactiae*	No identification	V
Abiotrophia defectiva	*Streptococcus mitis/oralis*	*Abiotrophia defectiva*	M
Staphylococcus epidermidis	*Staphylococcus auricularis*	*Staphylococcus epidermidis*	M
Staphylococcus epidermidis	*Staphylococcus warneri*	*Staphylococcus epidermidis*	M
Staphylococcus haemolyticus	*Staphylococcus warneri*	*Staphylococcus haemolyticus*	M
Staphylococcus hominis	*Staphylococcus saprophyticus*	*Staphylococcus hominis*	M
Staphylococcus saprophyticus	*Staphylococcus hominis*	*Staphylococcus saprophyticus*	M
Staphylococcus saprophyticus	*Staphylococcus warneri*	*Staphylococcus saprophyticus*	M
Streptococcus anginosus	*Streptococcus constellatus*	*Streptococcus anginosus*	M
Streptococcus anginosus	*Streptococcus gordonii*	*Streptococcus anginosus*	M
Streptococcus constellatus	*Streptococcus intermedius*	*Streptococcus constellatus*	M
Streptococcus constellatus	*Gemella morbillorum*	*Streptococcus constellatus*	M
Streptococcus gordonii	*Streptococcus sanguis*	*Streptococcus gordonii*	M
Streptococcus mitis	*Granulicatella elegans*	*Streptococcus mitis/oralis*	M
Streptococcus salivarius	*Streptococcus mitis*	*Streptococcus salivarius*	M
Staphylococcus pettenkoferi	*Staphylococcus auricularis*	*Staphylococcus capitis*	B
Staphylococcus pettenkoferi	*Staphylococcus auricularis*	No identification	B
Staphylococcus pettenkoferi	*Staphylococcus warneri*	No identification	B

**S. caprae* and *S. capitis* could not be differentiated by 16S rRNA gene sequencing.

424). Matching at species level was 95.0 % for staphylococci, 91.9 % for streptococci and 100 % for enterococci. Table 2 provides detailed results of discrepant results between the conventional method and the Vitek MS. Among 23 isolates with discrepant results between the conventional method and the Vitek MS, 14 isolates, including *S. epidermidis, Staphylococcus haemolyticus, Staphylococcus hominius, Staphylococcus saprophyticus, S. anginosus, Streptococcus constellatus, Streptococcus gordonii, Streptococcus salivarius, Streptococcus mitis* and *A. defectiva*, were correctly identified at species level with the Vitek MS but were proposed as different species in the same genera with the conventional method. In six isolates including *S. warneri, S. capitis, S. anginosus* and *S. dysgalactiae*, the conventional method correctly identified at the species level but the Vitek MS proposed multiple choices (LD) including reference identification or no identification. Of the three isolates of *S. pettenkoferi*, none were correctly identified by either the Vitek MS or conventional methods (Table 2).

DISCUSSION

MALDI-TOF MS has become a major revolution in the practice of bacteriology in clinical microbiology laboratories, and we expect this technology will soon replace most of the traditional identification methods due to its many benefits (Bizzini *et al.*, 2010; Benagli *et al.*, 2011). MALDI-TOF MS can perform accurate identification of bacteria using a small portion of a colony and at a low running cost (van Veen *et al.*, 2010; Neville *et al.*, 2011). Moreover, the time to final result is very fast compared to the conventional method, and the ability to make identifications directly from positive blood cultures could further enhance the quality of patient management (Christner *et al.*, 2010; Kaleta *et al.*, 2011; Yan *et al.*, 2011). Although the Vitek MS has only recently been launched, several recent studies have shown an enhanced accuracy for some bacterial species, such as *Streptococcus* species, and good performance without an initial extraction step (Dubois *et al.*, 2012; Fang *et al.*, 2012; Harris *et al.*, 2012; Marko *et al.*, 2012; Martiny *et al.*, 2012). Several studies have reported that the Vitek MS correctly identified 80.0 % to 93.2 % of routine isolates at species level (Dubois *et al.*, 2012; Harris *et al.*, 2012; Marko *et al.*, 2012; Martiny *et al.*, 2012). Compared to other systems, the analytical sensitivities of the Vitek MS and Biotyper were similar (Marko *et al.*, 2012; Martiny *et al.*, 2012). The overall accuracy can be different based on the distributions of organism groups and is generally higher in Gram-positive cocci than in *Enterobacteriaceae*, nonfermentative Gram-negative rods, and anaerobes (Dubois

et al., 2012; Harris *et al.*, 2012; Marko *et al.*, 2012; Martiny *et al.*, 2012). For Gram-positive cocci only, the reported accuracies of identification at species level using the Vitek MS were 92.1 % to 100.0 % for staphylococci, 87.3 % to 98 % for streptococci and 93.9 % to 100.0 % for enterococci, which were better than for other systems (Dubois *et al.*, 2012; Fang *et al.*, 2012; Harris *et al.*, 2012; Martiny *et al.*, 2012). Because the number of streptococci and enterococci were relatively limited in these previous studies (Dubois *et al.*, 2012; Martiny *et al.*, 2012), we focused on Gram-positive cocci that are routinely isolated in clinical microbiology laboratories. The Vitek MS correctly identified 97.9 % of the total Gram-positive cocci at species level in this study. According to organism groups, correct identification was at the species level for 97.2 % of the staphylococci, 97.8 % of the streptococci and 100 % of the enterococci. The overall good performance of the Vitek MS was in line with with the findings in previous studies (Dubois *et al.*, 2012; Harris *et al.*, 2012; Martiny *et al.*, 2012).

The Vitek MS showed good accuracy for identification of streptococci. All isolates of *S. pneumoniae*, 98.1 % of beta-haemolytic streptococci and 96.7 % of viridians streptococci were correctly identified to the species level. We confirmed the results of previous studies that showed better performance of the Vitek MS in identification of streptococci than other systems (Dubois *et al.*, 2012; Martiny *et al.*, 2012; Dubois *et al.*, 2013). The Vitek MS showed particularly excellent accuracy in identifying *S. pneumoniae* in our study and in previous studies. This is in contrast to other MALDI-TOF MS systems in which accuracies were lower in *S. pneumoniae* and viridans streptococci (Seng *et al.*, 2009; Bizzini *et al.*, 2010; Cherkaoui *et al.*, 2010; van Veen *et al.*, 2010; Benagli *et al.*, 2011; Neville *et al.*, 2011). In our tests, the Vitek 2 system misidentified some anginosus group or viridans streptococci isolates but the Vitek MS correctly identified most of these (Table 2). Although the Vitek MS could not differentiate some organisms within the same group (e.g. *S. mitis* and *S. oralis*), by allowing quick and reliable identification of streptococci, the Vitek MS can resolve the limitations of identification of conventional methods, especially in the anginosus group and viridans streptococci. As noted in a previous study (Harris *et al.*, 2012), the Vitek MS also identified uncommon organisms such as *A. defectiva*, which could not be identified by conventional methods (Table 2). The Vitek MS showed LD results for *S. anginosus/constellatus* in two *S. anginosus* isolates, and this LD was also previously noted in some of the anginosus group of streptococci in other studies (Dubois *et al.*, 2012). One isolate of *S. dysgalactiae* could not be identified by the Vitek MS, but it was correctly identified by the Vitek 2 system. As previously noted, the LD results to the subspecies level for *S. dysgalactiae* subsp. *dysgalactiae* and subspecies *equisimilis* were also noted in our study (n = 10) due to the resolution limit of the system itself (Dubois *et al.*, 2012). With 16S rRNA gene sequencing, these isolates could be identified at subspecies level as *S. dysgalactiae* subsp. *equisimilis*. For enterococci, both the Vitek MS and Vitek 2 systems correctly identified all isolates. Excellent performance of the Vitek MS for identification of enterococci was also noted in other studies (Dubois *et al.*, 2012; Fang *et al.*, 2012; Harris *et al.*, 2012).

With regard to CoNS, the overall correct species identification was 95.9 % and was lower than for *S. aureus* (100.0 %). The Vitek 2 system misidentified some CoNS isolates including *S. epidermidis*, *S. haemolyticus*, *S. hominis* and *S. saprophyticus*, which were correctly identified by the Vitek MS. Clearly, the Vitek MS demonstrated better performance than the conventional method, as have other MALDI-TOF MS systems (Dupont *et al.*, 2010; van Veen *et al.*, 2010). However, the Vitek MS also gave LD results including species of different genera such as *N. gonorrhoeae* and *P. buccalis* in two *S. warneri* isolates and gave no identification in one *S. capitis* and three *S. pettenkoferi* isolates. Compared to *S. aureus* and *S. epidermidis*, lower accuracies in other CoNS isolates were also noted in other studies and this needs to be improved through expanding the spectral databases. In particular, all isolates of *S. pettenkoferi* were shown to be misidentified or unidentified in both the Vitek MS and Vitek 2 systems because the species were not included in the databases of either system. Infections due to *S. pettenkoferi* have rarely been reported and identification should be possible through molecular methods in all cases (Trülzsch *et al.*, 2002; Loïez *et al.*, 2007; Song *et al.*, 2009). In a previous study, *S. pettenkoferi* was misidentified using the Vitek MS and SARAMIS databases, but not with the Biotyper (Martiny *et al.*, 2012). Among four *S. warneri* isolates, two isolates were identified at above genus level (LD results) by the Vitek MS, but the Vitek 2 system correctly identified these isolates at species level. The database of CoNS such as *S. pettenkoferi* and *S. warneri* should be improved in the Vitek MS.

Several limitations of this study should be mentioned. First, we selected well-characterized, aerobic Gram-positive cocci during the study period. We planned to include similar numbers of each species but the number of rare species was limited during the study period. Thus, accuracy of MALDI-TOF Vitek MS system could be overestimated. Second, 16S rRNA gene sequencing was performed in discrepant isolates between the Vitek MS and conventional identification or when the results of conventional identification were not certain or needed confirmation. Although we could assume that correct identification was obtained when there is agreement between two systems for practical reasons, concordance does not necessarily equate strictly to accuracy.

In conclusion, the MALDI-TOF Vitek MS system is a reliable method for the identification of most relevant Gram-positive cocci isolated in the clinical laboratory. This system can reduce turnaround time at low consumable cost and improve the identification of some species, such as streptococci, compared to conventional methods. Expanding the database, especially for CoNS, is needed to further enhance the performance of the Vitek MS.

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